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Specialized pollination mechanism, insect-pollination, autochory and anemochory in *Aganosma cymosa* (Roxb.) G. Don (Family Apocynaceae: sub-family Apocynoideae)

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ABSTRACT

Aganosma cymosa is a semi-evergreen woody climber. It is a massive bloomer for a short period. The flowers are white, nectariferous, pleasantly fragrant and hermaphroditic with a specialized pollination mechanism that is adapted for cross-pollination. The foragers included a wasp, flies, butterflies and a hawk moth. Among these, the flies have no role in pollination while all other insects effect either geitonogamy or xenogamy. Fruit set in open-pollinations does not exceed 4% of which 4.5% is 2-follicled and 0.5% is 1-follicled indicating that the plant is most likely a self-incompatible and obligate out-crosser and 1-follicled fruits represent only one of the ovaries of the flower. The white eye bird also visits the flowers for nectar but it simply steals the nectar causing damage to the sexual apparatus and flower fall. Seed dispersal modes include autochory and anemochory, the former mode ensures short-distance dispersal while the latter mode ensures long-distance dispersal for extending its distribution range.

Keywords: *Aganosma cymosa*, specialized pollination mechanism, hermaphroditism, cross-pollination, insect-pollination, autochory, anemochory.

1. INTRODUCTION

In tropical Apocynaceae species, the flowers display a complex pollination mechanism adapted for out-crossing (Fallen 1986). In most of the species of this family, the style head is segmented into an apical non-receptive area that receives self-pollen from the introrse anthers and forms a pollen chamber together with the anthers, a stigmatic receptive area at the base and a medium secretory area that produces a sticky mucilaginous substance that glues to the

tongue or proboscis of the visitors while retracting after nectar collection (Schick 1980, 1982). In Apocynaceae flowers, nectar is the only floral resource available for foragers (Fallen 1986). In this family, field-based information on plant-pollinator interactions is scarce (Alberts and van der Maesen 1994). However, the documented information shows that hermaphroditic flowers are common and self-compatibility is rare in this family (Rowley 1980). Many plant species depend on pollinators to achieve high reproductive success (Proctor et al. 1996). Insects are the main pollinators in this family. The large-flowered species of Apocynaceae have been speculated to have evolved to attract pollinating insects such as butterflies, bees, bumblebees and wasps with long and strong mouth parts which enable them to probe and gather nectar from the base of the flowers. Further, they are also visited by non-pollinating birds that steal nectar (Schick 1982; Lopes and Machado 1999; Alberts and van der Maesen 1994).

The genus *Aganosma* belongs to the sub-family Apocynoideae and family Apocynaceae. Its name is derived from *aganos* meaning mild and *osme* meaning smell, together indicating scent of flowers. The number of species assigned to this genus has been reported variously by different authors. Xiang Hua (1995) reported that it has about 12 species distributed in tropical and sub-tropical parts of Asia. Middleton (1996) documented that this genus has eight species distributed from India to China and southwards to the Philippines and Western Indonesia. Lemmens and Bunyapraphatsara (2003) reported that *Aganosma* comprises 8 species distributed from India and Sri Lanka through Indo-China, southern China, Thailand and Western Malesia to the Philippines and the Moluccas. These reports indicate that the number of species assigned to *Aganosma* is yet to be evaluated for ratification. Among the species of this genus, *A. cymosa* is widely reported in enumeration studies in its areas of distribution. Xiang Hua (1995) reported that *A. cymosa* is distributed in India, Bangladesh, Myanmar, Thailand, Cambodia, Vietnam and Laos. Swapna Reddy and Ramya Kuber (2020) reported that *A. cymosa* is restricted to the peninsular region in India and Bengal where it grows as a climbing shrub. This twining shrub is used in the treatment of bronchitis, leprosy, skin diseases, ulcers, arthritis, purulent discharges from ears, eye diseases, mouth diseases, fever, and also as a sedative. Lemmens and Bunyapraphatsara (2003) reported that *Aganosma* species are pollinated by insects such as bees and flies but details of pollinating behavior is not provided by them. This state of information on the genus *Aganosma* indicates that none of the species assigned to this genus have been investigated for their pollination ecology. Therefore, the present study is contemplated to describe certain aspects of floral biology, pollination mechanism and pollinators of *Aganosma cymosa* (Roxb.) G. Don. This information is useful to initiate studies on all other species of *Aganosma* throughout their distribution range.

2. MATERIALS AND METHODS

The semi-evergreen woody climber, *Aganosma cymosa* growing in Japalitheertham and Akasaganga sites of Tirumala Hills (13.42'2.6280 N, 79^{0}2o'21.0048 E) in Chittoor District, Andhra Pradesh, India, were used for study during July 2018 to November 2019. The host tree is *Bridelia* sp. (Phyllanthaceae) at these sites for *A. cymosa*. The aspects investigated included the plant phenology, flowering season, flower morphology and biology, pollination mechanism, forage collection schedule, and activity of foragers involved in pollination, fruiting and seed dispersal aspects. The anthesis schedule, anther dehiscence timing and stigma receptivity duration were observed in the field. The methods described in the book of Dafni et al. (2005) were used for nectar measurement and stigma receptivity duration. The number of flowers used for recording fruit set rate in open-pollinations was 75; they were tagged and followed for four weeks to note the number of fruits formed and calculate the percentage of fruit set. The fruit is either 2- or 1-follicled and the percentage of each follicle type was also calculated separately. Flower foragers collecting pollen or nectar were carefully examined for their flower probing behavior effecting pollination. The duration of fruit development, seed production and dispersal mode were also observed in the field.

3. OBSERVATIONS

The plant and flowering:

The plant is a semi-evergreen twining woody latex producing climber. It germinates at the ground, roots permanently in soil and climbs with its stem to reach the tree canopy which is represented by *Bridelia* sp (Figure 1a). The leaves are petiolate, simple, broadly ovate with rounded base and acuminate apex and borne opposite to each other. The flowering season falls between August and September with profuse flowering during 2nd week of September (Figure 1b). An individual plant flowers for about 2-3 weeks only. The flowers are borne in many-flowered cymes or clusters at the end of branches. The cymes are pedunculate and positioned above the foliage making them very prominent which attract pollinating foragers from a long distance. Individual cymes flower for 4-5 days (Figure 1c). Mature buds open sporadically day long but most of them open during 0600-0800 h (Figure 1d-h).

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Flower morphology and biology:

The flowers are pedicellate, 13.4 ± 8.1 mm long, 12.4 ± 6.2 mm wide, white, pleasantly fragrant, actinomorphic and bisexual. Calyx consists of five green, free narrowly elliptic spreading sepals and each sepal is 7.9 ± 0.6 mm long, 1.5 ± 0.4 mm wide and pubescent on both sides. The corolla is tubular, 5.6 ± 4.8 mm long and extended into five lobes; the tubular portion is light green outside and white inside while the lobes are completely white, spreading and twisted to the right side. Each lobe is 6.1 ± 0.5 mm long and 2.6 ± 0.4 mm wide. The stamens are 5, white and included in the lower portion of the corolla tube; the filaments are short and the anthers (4.6 ± 0.5 mm) are fertile in the upper half and sterile in the lower half. The sterile half of anthers is enlarged and this portion is with lignified guide rails laterally and sagittate appendages at the base (Figure 1i) but adnate to the style head at two points. The anthers dehisce longitudinally during anthesis. The pollen grains are monads, smooth, tricolporate, 40.6 ± 5.31 μm, 838.1 ± 79.4 per anther, 4,190.5 ± 397.2 per flower; the pollen-ovule ratio is 114.4:1. The disc is a continuous flat topped 5-dentate ring but it is longer than ovary. The ovary has 2 separate carpels united into a common glabrous light green style and pistil head is ovoid with a 2-cleft wet stigmatic apex which attains receptivity after anthesis and remains functional until the evening of the next day. Each carpel has numerous ovules which vary from 14 to 22 (18.15 ± 1.59). Nectar is secreted at the base of the corolla, covered above by sexual apparatus placed at the corolla throat and it amounted to 3-4 μl per flower.

Thrips activity and pollination:

Mature buds contained different stages of thrips and emerged out upon anthesis. After anthesis, the thrips moved in and out flowers through corolla throat, visited flowers of the same branch/tree. They collected pollen and nectar and carried pollen on their body as they were found coated all over with pollen; this foraging activity was considered to be effecting vector-mediated autogamy/geitonogamy. As they were resident foragers, they continuously moved between flowers to feed on pollen and nectar during day time and hence were treated to be resident pollinators.

Pollination mechanism:

The flowers present a specialized pollination mechanism. The style head together with over-arched anthers form the sexual apparatus (Figure 1j). In the style head, the apical bi-lobed portion, sticky mucilaginous thinner portion and the pollen receiving portion. The apical lobed portion is the seat of self-pollen deposition while the pollen receiving portion is the seat for cross-pollen reception. These two portions are separated by the mucilaginous portion. The flower visitors insert their tongue/proboscis into the corolla tube to access nectar situated at the corolla base. While doing so, tongue or proboscis do not come in contact with the apical portion where self-pollen is deposited but scrapes the base of the style head. Then, cross-pollen carried by the flower visitor on its tongue portion or proboscis is deposited on to the pollen receiving portion of the style head. The tongue or proboscis then passes sticky mucilaginous thinner portion and the apical portion in quick succession capturing the self pollen deposited in the apical portion. This sequential process associated with the probing and departing behavior of the forager results in geitonogamous or xenogamous pollination if the forager had pollen from the previously visited flowers and also simultaneously or in quick succession transfers self-pollen by the forager on to its tongue or proboscis for effecting either geitonogamy or xenogamy in the flowers to be visited by the forager.

Insect activity and pollination:

The flowers were foraged during day time from 0800-1700 h with maximum foraging visits at 1000 h by insects and a bird species (Figure 3,4). The foragers included the wasp, *Vespa bicincta* (Figure 2a), flies *Chrysomya megacephala* (Figure 2b), *Sarcophaga orchidea* (Figure 2c), *Eristalinus arvorum* (Figure 2d), butterflies *Papilio polytes* (Figure 2e), *Pachliopta hector* (Figure 2f), *P. clytia* (Figure 2g) (Papilionids), *Tirumala septentrionis* (Figure 2h), *Hypolimnas bolina* (Figure 2i) (Nymphalids), the diurnal hawkmoth *Macroglossum gyrans* (Figure 2j), and the white eye, *Zosterops palpebrosa* (Figure 2k,l). Of these, the flies collected pollen while all other insects collected nectar. Of the total foraging visits made by insects, the wasp visits constituted 14%, flies 30% and butterflies and the hawk moth 56% (Figure 5). Among these foragers, flies as exclusive pollen collectors appeared to facilitate self-pollen deposition in the apical portion of the style head. The flies never made any attempt to probe through the corolla tube to collect nectar and hence they were treated as sole pollen collectors without any role in pollination. All other insects as exclusive nectar collector probed through the corolla tube to collect nectar and in this probing process, they were found to be effecting either geitonogamous or xenogamous pollinations. The white eye bird probed the flowers by inserting its bill into the corolla tube to collect nectar but it usually punctured the corolla tube causing damage to the sexual apparatus and sometimes, the flowers fell off indicating that this bird is a nectar robber and flower destroyer and disallows the flowers probed by it to participate in the sexual reproduction.

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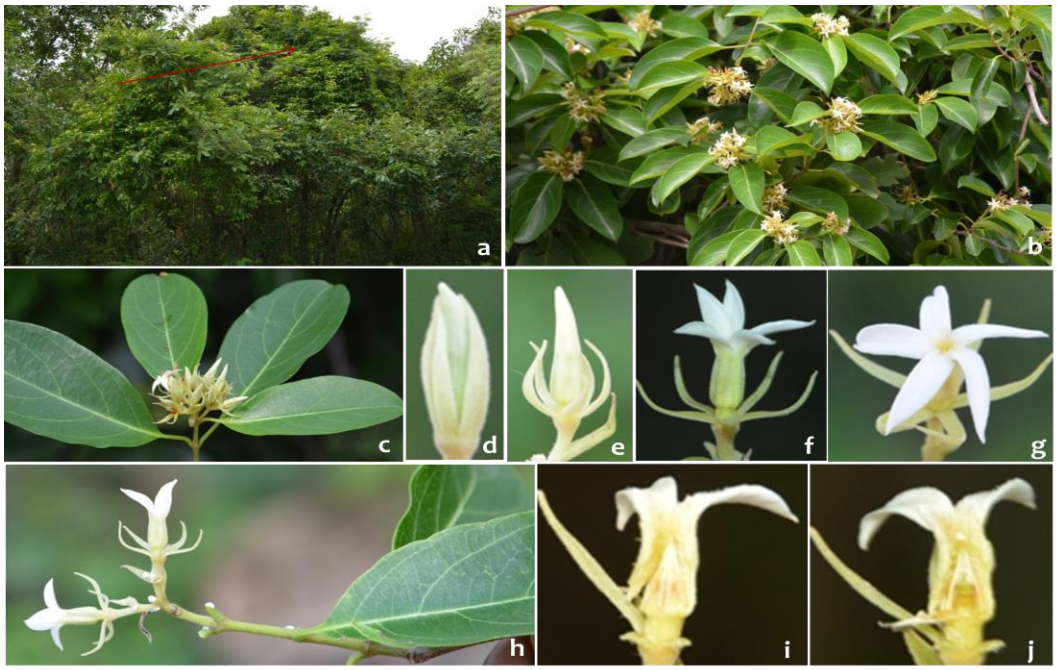

Figure 1. *Aganosma cymosa*: a. Woody climber, *Bridelia* sp. host tree, b. Flowering phase, c. Twig with flowering inflorescence, d-g. Anthesis stages, h. Flowers, i. Cut flower showing sagittate anthers, j. Cut flower showing pistil which is enclosed by anthers.

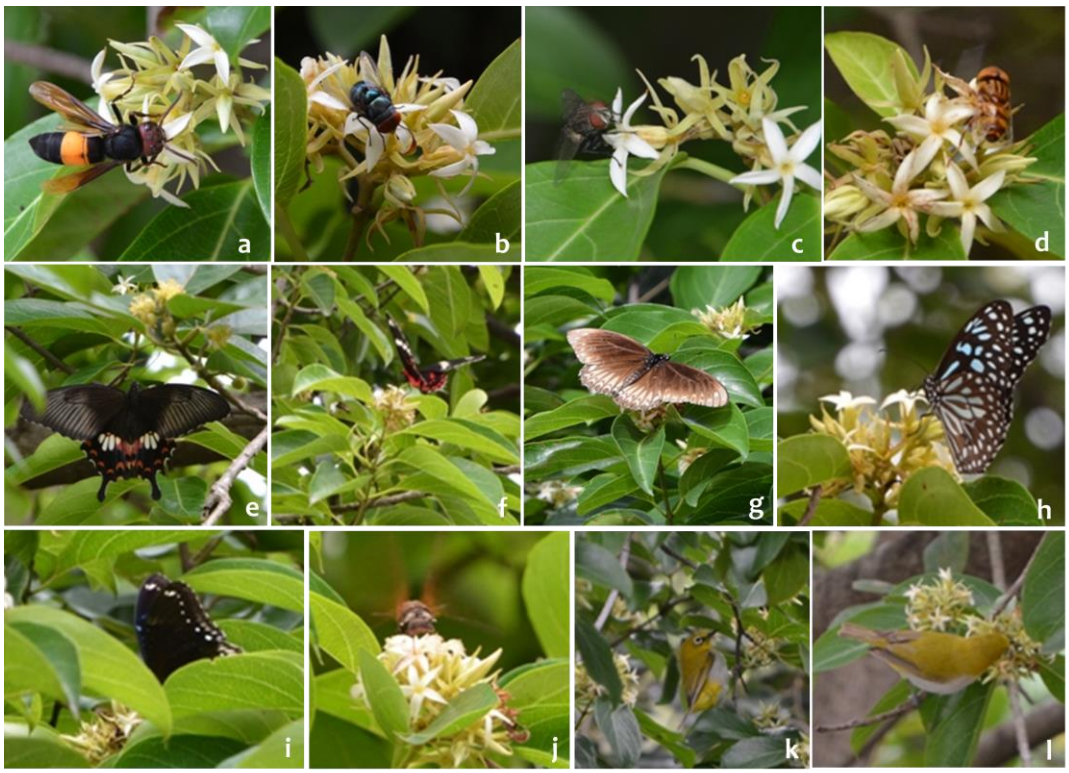

Figure 2. *Aganosma cymosa*: a. *Vespa bicincta* collecting nectar, b. *Chrysomya megacephala* collecting pollen, c. *Sarcophaga orchidea* collecting pollen, d. *Eristalinus arvorum* collecting pollen, e-h. Papilionid butterflies collecting nectar– e. *Papilio polytes,* f. *Pachliopta hector*, g. *Pachliopta clytia* (female), h. & i. Nymphalid butterflies collecting nectar– h. *Tirumala septentrionis*, i. *Hypolimnas bolina* (female), j. Diurnal hawkmoth, *Macroglossum gyrans* collecting nectar, k. & l. *Zosterops palpebrosa* collecting nectar.

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Figure 3. *Aganosma cymosa*: a. Fruiting phase, b. Close-up view of follicles, c. A pair of follicles formed from a fertilized flower, d. A single follicle formed from a fertilized flower.

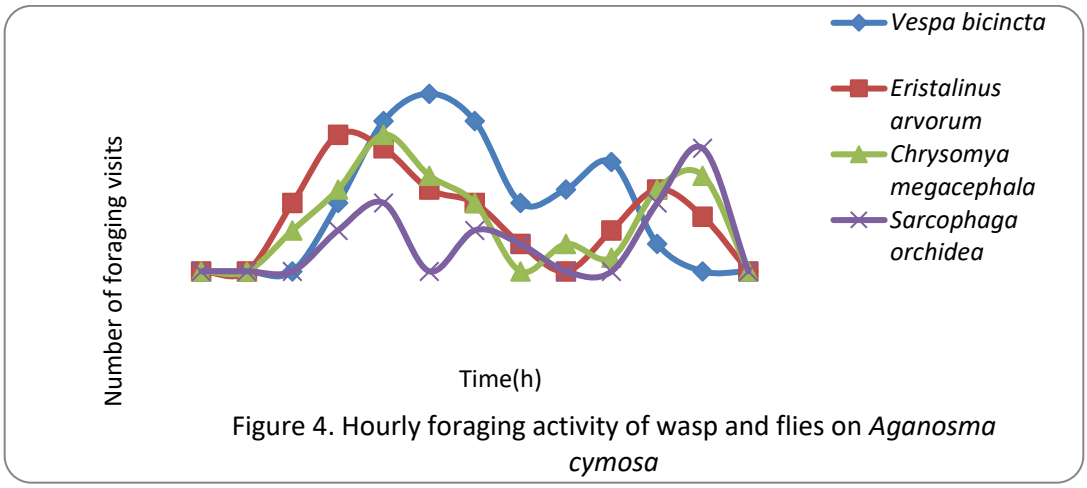

Figure 4. Hourly foraging activity of wasp and flies on *Aganosma cymosa*

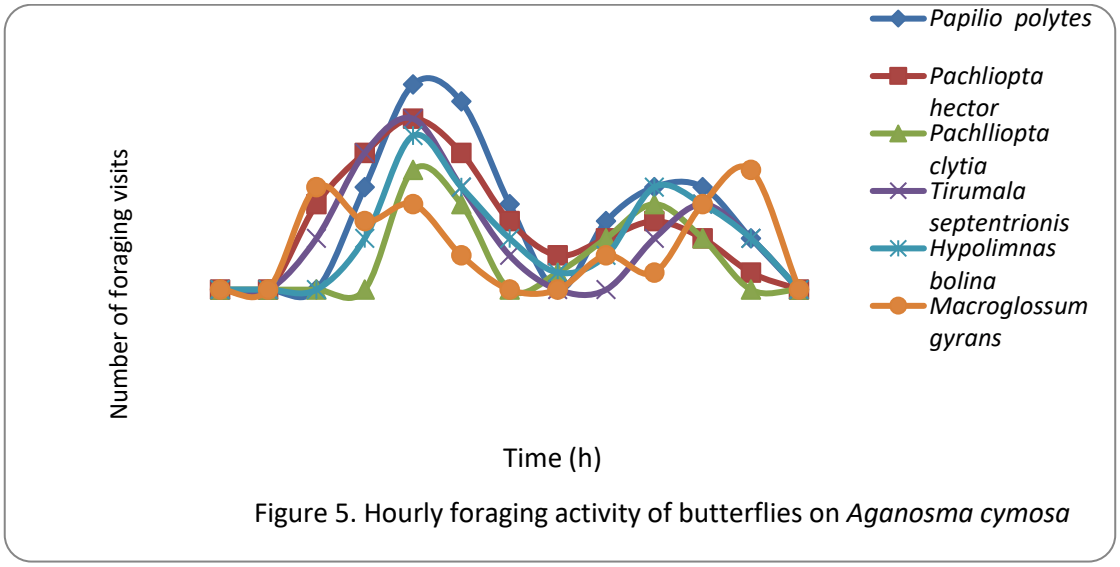

Figure 5. Hourly foraging activity of butterflies on *Aganosma cymosa*

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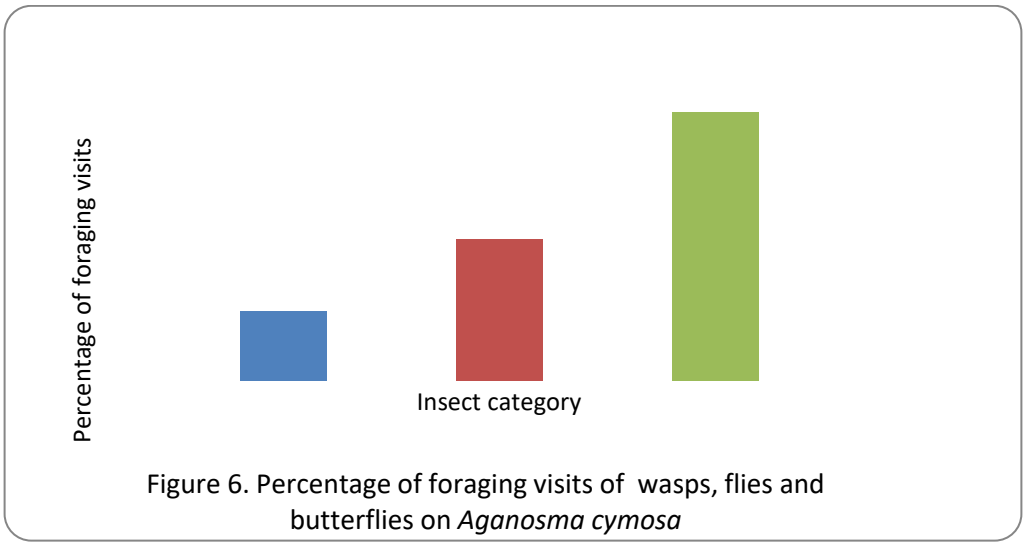

Figure 6. Percentage of foraging visits of wasps, flies and butterflies on *Aganosma cymosa*

Fruiting and seed dispersal:

Fruit is a dehiscent follicle-type and mature within 5-6 weeks (Figure 3a, b). A pair of linear cylindric divergent, pubescent, 16.02 ± 3.29 cm long grey colored follicles attached to a common stalk is produced from the fertilized flowers (Figure 3c). Each follicle is produced from one carpel and contains several 15 x 4 mm, yellow, narrowly elliptical, flattened seeds tipped with coma characterized by numerous 2.5-4.5 cm long hairs. The fruiting rate in open-pollinations is only 4% of which 0.5% represented single follicular fruits (Figure 3d). Follicles dehisce longitudinally to disperse seeds which are either simultaneously or subsequently dispersed by wind.

4. DISCUSSION

Woody climbers are dynamic life-forms, more prominent and characteristic in tropical forests of Tropical America, Africa and Asia (Gentry 1991; Schnitzer and Bongers 2002; Mascaro et al. 2004). They germinate at the ground level, root deep in soil and climb host trees with their specialized climbing structures to reach the canopy cover (Gerwing 2004). In the present study, *Aganosma cymosa* is a semi-evergreen twining woody climber and producer of latex. It starts its growth from the ground level, roots deep into the soil and climbs with its twining stem to reach the tree canopy. It uses a member of Phyllanthaceae, *Bridelia* sp. as a host tree which is completely covered on the top by *A. cymosa*. It displays a short period of flowering but blooms massively making its flowers very prominent above foliage. Further, the flowers being white and pleasantly fragrant are quite attractive from a longer distance to the flower foragers.

In tropical Apocynaceae species, the flowers display a complex pollination mechanism adapted for out-crossing (Fallen 1986). The style head is segmented into an apical non-receptive area that receives self-pollen from the introrse anthers and forms a pollen chamber together with the anthers, a stigmatic receptive area at the base and a medium secretory area that produces a sticky mucilaginous substance that glues to the tongue or proboscis of the visitors while retracting after nectar collection (Schick 1980, 1982). In this study, *A. cymosa* displays such a pollination mechanism with the style head over-arched by introrse anthers. In the style head, the apical portion is a non-receptive area but serves as a seat for self-pollen received from introrse anthers. The receptive area at the base of style head receives pollen from the tongue or proboscis of nectar-probing foragers and gets pollinated either geitonogamously or xenogamously depending on the source of pollen brushed off by the probing foragers. After nectar collection, the proboscis or tongue of the probing forager passes the mucilaginous portion and then apical portion of the style head in quick succession gathering self-pollen from the non-receptive apical portion spontaneously on its tongue or proboscis for subsequent transfer to the flowers of the same or a different individual. Therefore, the pollination mechanism functional in *A. cymosa* is adapted for achieving cross-pollination in preference to self-pollination.

Proctor et al. (1996) reported that many plant species depend on pollinators to achieve high reproductive success. Different authors reported that insects are the main pollinators in Apocynaceae. In this family, the large-flowered species have been considered to have evolved to attract pollinating insects such as butterflies, bees, bumblebees and wasps with long and strong mouth parts which enable them to probe and gather nectar from the base of the flowers. Further, they are also visited by non-pollinating birds that steal nectar (Schick 1982; Lopes and Machado 1999; Alberts and van der Maesen 1994). Lemmens and Bunyapraphatsara (2003) reported that *Aganosma* species are pollinated by insects such as bees and flies but details of pollinating

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behavior is not provided by them. In this study, *A. cymosa* is foraged by a wasp, flies, butterflies and a hawk moth. Among these foragers, the flies while collecting pollen facilitate self-pollen deposition in the apical non-receptive portion of the style head but do not effect pollination. All other insects while collecting nectar effect either geitonogamous or xenogamous pollination; however, the wasp and the hawk moth are effective pollinators due to its efficient probing for nectar while butterflies are not as effective as the wasp and the hawk moth due to their passive probing behavior. In open pollinations, *A. cymosa* despite being foraged and pollinated by different insects is able to set fruit to the extent of 4% only indicating that this woody climber is most likely a self-incompatible and obligate out-crosser. Out of this fruit set, 4.5% represents 2-follicled fruits and 0.5% represents 1-follicled fruits indicating the pollination and fertilization of ovules of only one ovary; this situation also speaks about the probing of pollinators from one side of the corolla tube that results in the pollination and subsequent fertilization of ovules in one of the ovaries of the flower. Further, the white eye bird resorts to puncture the corolla tube causing damage to the sexual apparatus and even falling of flowers indicating that this bird is a nectar robber and flower destroyer. As a result, the flowers probed by this bird fail to take participation in the production of fruits/seeds and hence the bird's foraging activity has a negative impact on the sexual reproduction of *A. cymosa*.

Sarvalingam and Rajendran (2016) reported that *A. cymosa* is anemochorous in the Western Ghats of Tamilnadu. In this study, it is found that *A. cymosa* is first autochorous as the mature and dry follicles dehisce longitudinally to disperse seeds. The seeds thus released from the follicles are carried away by wind. Therefore, both autochory and anemochory are functional in this climber, the former mode ensures short-distance dispersal while the latter mode ensures long-distance dispersal for extending its distribution range.

Swapna Reddy and Ramya Kuber (2020) reported that *A. cymosa* is used in the treatment of bronchitis, leprosy, skin diseases, ulcers, arthritis, purulent discharges from ears, eye diseases, mouth diseases, fever, and also as a sedative. Since this climber is used for the treatment of human diseases in traditional medicine, it can be exploited as a medicinal plant in order to control its proliferation on tree canopy.

5. CONCLUSIONS

Aganosma cymosa is a semi-evergreen woody climber. It blooms massively for a short period. The flowers being white, nectariferous and pleasantly fragrant are quite attractive from a longer distance to the flower foragers. The flowers are hermaphroditic and have a specialized pollination mechanism that is adapted for cross-pollination. The foragers included a wasp, flies, butterflies and a hawk moth. Among these, the flies while collecting pollen facilitate self-pollen deposition in the apical non-receptive portion of the style head without effecting pollination. All other insects while collecting nectar effect either geitonogamy or xenogamy. Fruit is either 2- or 1-follicled. Fruit set in open-pollinations does not exceed 4% of which 4.5% is 2-follicled and 0.5% is 1-follicled indicating that the plant is most likely a self-incompatible and obligate out-crosser and 1-follicled fruits represent only one of the ovaries of the flower. The white eye bird also visits the flowers for nectar but it simply steals the nectar causing damage to the sexual apparatus and flower fall. Seed dispersal modes include autochory and anemochory, the former mode ensures short-distance dispersal while the latter mode ensures long-distance dispersal for extending its distribution range.

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Authors' contributions

All authors contributed equally.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for species collection & identification.

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Data and materials availability

All data associated with this study are present in the paper.

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